CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
METAFLUMIZONE

Chemical Code # 5935 Tolerance # 53000

1 August 2006

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap, no adverse effects
Chronic toxicity, dog:	No data gap, no adverse effects
Oncogenicity, rat:	No data gap, no adverse effects
Oncogenicity, mouse:	No data gap, no adverse effects
Reproduction, rat:	No data gap, no adverse effects
Teratology, rat:	No data gap, no adverse effects
Teratology, rabbit:	No data gap, no adverse effects
Gene mutation:	No data gap, no adverse effects
Chromosome effects:	No data gap, possible adverse effects
DNA damage:	No data gap, no adverse effects
Neurotoxicity:	No data gap, no adverse effects

Toxicology one-liners are attached.

All record numbers through 222586 were examined.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.

File name: T060912, prepared by H. Green and T. Moore

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

COMBINED, RAT

** 53000-0051; 222561; "90-Day/24-Month Toxicity and Oncogenicity Study with BAS 320I in Rats Via Oral Gavage Administration"; (C.M. Kelly; Huntingdon Life Sciences, East Millstone, NJ; Project ID. 00-2655; 12/3/03); Eighty Sprague-Dawley rats/sex/group were dosed orally by gavage with 0 (vehicle: aqueous 0.5% carboxymethylcellulose), 30, 60 or 300 mg/kg/day of BAS 320 I technical (lot no. AC 12372-85; purity: 96.3% (E:Z isomer ratio 92:8)) for 24 months. The 300 mg/kg treatment level was adjusted to 200 mg/kg/day for the females after 2 weeks of treatment due to excessive body weight loss and reduced food consumption. The treatment period for the 300 mg/kg males were terminated after 23 months of treatment due to excessive mortality for the control animals. The mean body weights of the males in the 300 mg/kg group and the females in the 60 and 200/300 mg/kg groups were lower than those of the control animals at various time points during the first three months of the treatment ($p<0.01$ or 0.05). This lower mean body weight persisted throughout the remainder of the study for the females. Food consumption was lower for the females in the 200/300 mg/kg group during the first weeks of the study. In the hematology evaluation after 3 months of treatment, the mean hemoglobin concentration and red blood cell count were reduced for the males in the 300 mg/kg group ($p<0.01$ or 0.05). The mean reticulocyte count was elevated for this group ($p<0.01$). The hematology values demonstrated no consistent treatment-related effects throughout the remainder of the study. The Functional Observational Battery and motor activity assessments after 12 weeks of treatment did not reveal any treatment-related effects. No treatment-related effect was noted in the ophthalmology, clinical chemistry or urinalysis. No treatment-related effects on organ weights were evident in the necropsy examination. In the histopathology examination, centrilobular hepatocellular hypertrophy in the liver was evident for the males in the 60 and 300 mg/kg groups (0: 0/10 vs. 60: 2/10, 300: 9/10). This effect persisted throughout the remainder of the study (0: 0/80 vs. 60: 20/80, 300: 49/80, $p<0.05$). An increased incidence of basophilic alteration in the hepatocytes was also noted for the males in the 300 mg/kg group from 12 months of treatment through to the termination of the study (0: 9/80 vs. 300: 20/80, $p<0.05$). The females in the 200/300 mg/kg group also demonstrated an incidence of centrilobular hepatocellular hypertrophy by the termination of the study (0: 0/80 vs. 200/300: 13/80, $p<0.05$). **No adverse effect indicated. Subchronic Oral Toxicity NOEL:** (M/F) 30 mg/kg/day (based upon the incidence of centrilobular hepatocellular hypertrophy in the liver of the males of the 60 mg/kg treatment group and the lower mean body weight of the females in the 60 mg/kg group); **Chronic Oral Toxicity NOEL:** (M) 30 mg/kg/day (based upon the incidence of centrilobular hepatocellular hypertrophy in the liver of the males of the 60 mg/kg treatment group), (F) 60 mg/kg/day (based upon the incidence of centrilobular hepatocellular hypertrophy in the liver of the females in the 200/300 mg/kg group); **oncogenicity was not evident.**. **Study acceptable.** (Moore, 6/12/06)

CHRONIC TOXICITY, RAT

See Combined, Rat above.

CHRONIC TOXICITY, DOG

53000-0048; 222558; "BAS 320 I - Sub-chronic/Chronic Oral Toxicity Study in Beagle Dogs, Administration via Gelatin Capsules for 3 and 12 Months"; (U. Kaspers, K. Deckardt, W. Kaufmann, B. van Ravenzwaay; Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany; Project ID. 43D0071/01085; 1/14/04); Five beagle dogs/sex/group were dosed orally with 0, 6, 12, 30 or 60 mg/kg/day of BAS 320 I Technical (batch no. PF-01-02-Ve-1-3, purity: 96.9%) in gelatin capsules for 12 months. The dose level for the high dose group was first adjusted down to 40 mg/kg/day on day 49 and then readjusted to 30 mg/kg/day on day 245 due to animals in this treatment group exhibiting body weight loss and reduced food consumption. One male and two females in the 60/40 mg/kg group were euthanized on day 57. One male and one female in this group were euthanized on days 250 and

226, respectively. Two females in the 30 mg/kg group were euthanized on study days 215 and 237. Although there was no statistically-significant effect on the body weight and food consumption data, individual animals exhibited a significant reduction in both their body weight and food consumption. The mean red blood cell count, hemoglobin levels and hematocrit for both sexes in the 30 mg/kg and 60/40/30 mg/kg groups were lower than the values for the controls over the course of the study (NS or $p<0.05$). These lower values were reflected in a lower calculated MCHC for both sexes in these groups (NS, $p<0.05$ or 0.01). Although the MCHC values for both sexes in the 6 and 12 mg/kg group were statistically different from those of the control at various times during the study, a dose response was not readily evident. In the clinical chemistry examination, the serum total bilirubin levels for both sexes in the 30 and 60/40/30 mg/kg groups were elevated at various times during the treatment period ($p<0.05$ or 0.01). In the necropsy examination, the mean absolute and relative spleen weights were greater for both sexes in the 30 and 60/40/30 mg/kg groups (NS). Although the mean absolute and relative liver weights were greater for males in all of the treatment groups, no dose-response effect was evident. In the histopathological examination, atrophy of the testes, prostate gland and thymus was noted for the animals in the 30 and 60/40/30 mg/kg groups which were euthanized during the study. A greater degree of pigment storage in the kidney was reported for the males in all of the treatment groups with evidence of a dose-related effect. For the females, this effect was less evident. **No adverse effect indicated. Chronic Oral Toxicity NOEL:** (M/F) 12 mg/kg/day (based upon the lower mean body weight and reduced food consumption demonstrated by individual animals in the 30 mg/kg group); **Study acceptable.** (Moore, 5/9/06)

ONCOGENICITY, RAT

See Combined, Rat above.

ONCOGENICITY, MOUSE

** 53000-0052; 222562; “18-Month Oncogenicity Study with BAS 320I in Mice Via Oral Gavage Administration”; (C.M. Kelly; Huntingdon Life Sciences, East Millstone, NJ; Project ID. 00-2656; 3/19/03); Sixty five CD-1 mice/sex/group were dosed orally by gavage with 0 (vehicle: aqueous 0.5% carboxymethyl cellulose), 100, 250 or 1000 mg/kg/day of BAS 320 I technical (lot no. AC 12372-85; purity: 96.3% (E:Z isomer ratio 92:8)) for 18 months. There was no treatment-related effect upon the survivability of the treated animals. The mean body weights and mean body weight gain of the 1000 mg/kg males was lower than that of the controls over the course of the study. There was no apparent treatment-related effect upon the mean food consumption of the treated animals. Although various hematology parameters of both sexes in the 1000 mg/kg group were statistically significantly lower at the 12 and 18 month treatment intervals, no biological significance could be assigned to these results. In the histopathological examination, an increased incidence of greater brown pigment was noted in the spleen of both sexes in the 1000 mg/kg group ((M) 0: 0/65 vs. 1000: 19/65, (F) 3/65 vs. 1000: 27/65, $p<0.05$). Although there was an increased incidence of inflammation of the visceral pleural/capsule and pleural thickening/adhesions and the presence of granulomatous inflammation in the mediastinal tissue for the males in the 1000 mg/kg group ($p<0.05$), these effects were attributed to injuries inflicted as a consequence of the gavage dosing regimen. **No adverse effect indicated. Chronic Oral Toxicity NOEL:** (M/F) 250 mg/kg/day (based upon increased pigmentation in the spleen of both sexes in the 1000 mg/kg group); **No oncogenicity evident.**. **Study acceptable.** (Moore, 6/20/06)

REPRODUCTION, RAT

**53000-0055 222565, “BAS 320 I - Two-Generation Reproduction Toxicity Study in Wistar Rats, Oral Administration (Gavage) (Including Amendment 1)”, (S. Schneider, *et al*., BASF Experimental Toxicology and Ecology, Ludwigshafen, Germany, Report Project No. 73R0071/01058, 24 February 2004. Amendment Date: 12 January 2005). 25 Wistar (CrlGlxBrlHan:WI) rats per sex per group received BAS 320 I by oral gavage at 0 (0.5% aqueous carboxymethylcellulose), 12, 30, and 75 mg/kg/day through the F1a generation. Due to maternal toxicity and pup mortality, all F1a pups were sacrificed. Subsequently, the study was restarted with lower mid and high dose levels using the original F0 parental animals. The revised dose levels were 0, 12, 20, and 50 mg/kg/day and the study continued through 2 generations (one litter

per generation). Treatment began 75 days before mating. Initial treatment levels (0, 12, 30, and 75 mg/kg/day) for F0 animals (F1a litter production). 1 mid dose and 11 high dose females (during premating), 1 mid-dose and 6 high-dose females (during gestation), and 5 high-dose females (during lactation) showed poor general states of health. Significant reductions in female bodyweight and food consumption during premating, gestation, and lactation were noted. High dose maternal bodyweight gains were also significantly reduced during premating and bodyweight gains for mid-dose females were significantly below control values during gestation days 0-7. One (4%), 0, 4 (16%), and 7 males (28%) at 0, 12, 30, and 75 mg/kg/day respectively failed to produce litters. This represented a significant reduction in the male fertility index at the high dose level vs concurrent controls and was outside of the historical control range. The female fertility index was also significantly reduced at the high dose level compared to concurrent controls and fell outside of the historical control range. 1 (4%), 0, 4 (16%), and 6 (25%) sperm-positive females at 0, 12, 30, and 75 mg/kg/day respectively did not deliver. The numbers of F1a pups that died and were cannibalized during the lactation period were significantly increased at 75 mg/kg/day (43 died, 7 cannibalized) compared to controls (1 and 0 respectively) and resulted from improper nursing behavior by some of the dams (a significant increase in the number of high dose pups with empty stomachs was noted at necropsy). The viability index (post-partum days 0-4, 93% vs 99% for controls) and lactation index (post-partum days 4-21, 70% vs 100% for controls) at the high dose level were both significantly decreased compared to controls. F0 Generation after Dose Level Reduction (0, 12, 20, and 50 mg/kg/day) and Production of F1b Litters. At F1a weaning, treatment levels for mid and high-dose F0 parental animals were reduced to 20 (from 30) and 50 (from 75) mg/kg/day. Parental animals were mated again with the same partners as for the F1a litters. Two mid-dose and 2 high-dose females died. Poor general state was noted for 1 mid-dose and 7 high-dose females during premating and for 3 high-dose dams during gestation. During lactation, one high dose female did not nurse her pups properly and none survived. Another dam showed poor general state, did not clean her pups after birth, did not cut the umbilical cords, nor consume the placentae and, consequently, all live pups died on the day of birth. Significantly lower group mean maternal bodyweights were recorded for high dose dams at the start of the F1b premating period compared to controls (as a result of the lower maternal bodyweights during production of the F1a litters) and continued throughout production of the F1b litters. Although, the corresponding maternal bodyweight gains during the same period for high dose females (50 mg/kg/day) were comparable to controls. There were no treatment-related effects on sperm parameters (motility, morphology, count) for F0 males. No treatment-related F0 necropsy and histopathology findings. The number of stillborn pups was significantly increased and the number of liveborn pups significantly decreased at 50 mg/kg/day compared to controls (live birth index was 98%, 98%, 99%, and 94% at 0, 12, 20, and 50 mg/kg/day respectively, (94% was slightly outside the historical control range (96% to 100%)). Post-natal survival was also impaired at the high dose level. 14 high-dose F1b pups died and 12 were cannibalized vs 1 and 0 for controls respectively (statistically significant). This was mainly due to 2 dams that failed to nurse their pups properly. Consequently, the viability index (post-partum days 0-4) and the lactation index (post-partum days 4-21) at the high dose level were both significantly decreased compared to controls (92% vs 100%). Low and mid-dose values were comparable to controls. Pup necropsy results were unremarkable. F1 Generation (0, 12, 20, and 50 mg/kg/day) and Production of F2a Litters. The male fertility index for F1 males was comparable to controls (96%, 96%, 92%, and 96% at 0, 12, 20, and 50 mg/kg/day respectively). F1 female reproduction and delivery parameters were not affected by treatment. The fertility index was 100%, 96%, 92%, and 96% at 0, 12, 20, and 50 mg/kg/day respectively. All pregnant dams had live F2 pups in their litters (gestation index of 100% for all groups). No treatment-related changes were noted for F1 parental clinical signs, food consumption, bodyweight, necropsy, and histopathology. F2a pup viability, development, mortality, sex ratios, clinical signs, bodyweights, and necropsy findings were not affected by treatment. Parental NOEL = 20 mg/kg/day (poor general state of some F0 females at 50 mg/kg/day during production of F1b litters). Reproductive NOEL = 20 mg/kg/day (reduced number of liveborn F1b pups, increased F1b pup mortality at 50 mg/kg/day). No adverse reproductive effects were indicated. Acceptable with deficiencies (dosing rationale). (Green and Leung, 9/1/06).

TERATOLOGY, RAT

**53000-0053 222563, "BAS 320 I - Prenatal Developmental Toxicity Study in Wistar Rats, Oral

Administration (Gavage)", (S. Schneider, *et al*., BASF Experimental Toxicology and Ecology, Ludwigshafen, Germany, Report Project No. 30R0071/01121, 24 February 2004). 25 time-mated female Wistar (CrlGlxBrlHan:WI) rats per group received BAS 320 I (96.9% metaflumizone) by oral gavage at 0 (0.5% aqueous carboxymethylcellulose), 15, 40, and 120 mg/kg on gestation days 6 through 19. There were no maternal deaths or treatment-related clinical signs. Group mean maternal food consumption was significantly reduced on gestation days 10 through 15 at 120 mg/kg/day relative to controls. Low and mid dose group food consumption was comparable to controls. Group mean maternal bodyweight was generally comparable to controls. Group mean maternal bodyweight gain was significantly reduced (54%) at 120 mg/kg/day on gestation days 6 through 8, and remained lower (not significant) than control values through the treatment period. Additionally, group mean corrected maternal bodyweight gain (terminal bodyweight on gestation day 20 minus weight of the unopened uterus minus bodyweight on day 6) or net weight change from GD 6 was significantly reduced at the high dose level compared to controls. No treatment-related fetal external or soft tissue malformations or variations were recorded. No treatment-related fetal skeletal malformations were noted. Fetal skeletal variations of the skull, the vertebral column, the ribs, the sternum, the limbs, and the pelvic girdle were noted in 95.6%, 92.9%, 95.4%, and 100% of fetuses/litter at 0, 15, 40, and 120 mg/kg/day respectively. Wavy rib was significantly increased in the low and mid dose groups as was the incidence of total skeletal variations at 120 mg/kg/day compared to controls. No teratogenicity. Maternal NOEL = 40 mg/kg/day (reduced food consumption and bodyweight gain). Developmental NOEL = 120 mg/kg/day (No signs of treatment-related teratogenicity). Acceptable. (Green and Leung, 9/11/06).

TERATOLOGY, RABBIT

**53000-0054 222564, "BAS 320 I - Prenatal Developmental Toxicity Study in Himalayan Rabbits, Oral Administration (Gavage)", (S. Schneider, *et al.*, BASF Experimental Toxicology and Ecology, Ludwigshafen, Germany, Report Project No. 40R0071/01116, 24 February 2004). 25 artificially-inseminated female Himalayan (Chbb:HM) rabbits received BAS 320 I (96.9% metaflumizone) by oral gavage at 0 (0.5% aqueous carboxymethylcellulose), 30, 100, and 300 mg/kg/day on gestation days 6 through 28. One high dose animal was sacrificed moribund. One control female and 2 high dose females aborted and were sacrificed. Lateral position, ataxia, and no defecation were reported for these animals prior to abortion/sacrifice. No treatment-related maternal effects were indicated for food consumption, bodyweight, bodyweight gain, or necropsy findings. Mean fetal weights were 5.5% and 7.4% lower (ns) than controls at 100 and 300 mg/kg/day respectively and the percentage of stunted fetuses/runts was increased at the high dose level vs controls (3.3%, 1.6%, 5.3%, and 13.8% at 0, 30, 100, and 300 mg/kg/day respectively). No treatment-related fetal external, visceral, or skeletal malformations were indicated. Mean percentages of fetuses/litter with soft tissue malformations were 3.7%, 4.8%, 3.2%, and 3.6% at 0, 30, 100, and 300 mg/kg/day respectively and mean percentages with skeletal malformations were 0%, 2.7%, 1.5%, and 0.7% at 0, 30, 100, and 300 mg/kg/day respectively. Maternal NOEL = 100 mg/kg/day (ataxia, no defecation, mortality at 300 mg/kg/day). Developmental NOEL 100 mg/kg/day (increased number of runts/stunted fetuses at 300 mg/kg/day). No teratogenicity. Acceptable. (Green and Leung, 9/11/06).

GENE MUTATION

**53000-0056 222566, "Bacterial Reverse Mutation Assay with An Independent Repeat Assay with BAS 320 I", (Valentine O. Wagner, III and Michelle L. Klug, BioReliance, Rockville, MD., Study No. AA27RH.502001.BTL, 15 May 2001). Triplicate cultures of *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 uvrA were exposed (direct plate incorporation) to BAS 320 I (96.3% metaflumizone), in the presence and absence of S9 rat liver fraction, at 0 (DMSO), 15, 50, 150, 1500, and 5000 µg/plate for 48 to 72 hours in two separate assays. Precipitates were noted on plates at 500 *µ*g/plate and above in assay one and at 150 *u*g/plate and higher in assay two. The background lawn was generally not effected by treatment. There was no increase in the number of revertants per plate. Acceptable (Green and Leung, 9/11/06).

**53000-0056 222567, 222568, "*In Vitro* Gene Mutation Test with BAS 320 I in CHO Cells

(HPRT Locus Assay)", (G. Engelhardt and E. Leibold, BASF Experimental Toxicology and Ecology, Ludwigshafen, Germany, Report Project No. 50M0071/014156, 26 August 2002). 500,000 Chinese hamster ovary cells (substrain K1) per flask were treated in duplicate, in the presence and absence of S9 rat liver fraction, at BAS 320 I (96.9% metaflumizone) concentrations of 0 (DMSO), 25, 50, 100, 156.3, 200, 312.5, 400, 625, 800, 1200, 1250, 2500, or 5000 μg/ml for 4 hours. Two trials were conducted. Expression time was 7 days followed by plating in selection medium with 6 thioguanine at 300,000 cells/flask with 12 flasks per treatment group (6 per replicate) for mutant selection. A reduction in the cloning efficiency was noted at 200 μg/ml and higher in the absence of S9. No increase in forward mutations. Acceptable. (Green and Leung, 9/11/06).

53000-0060 222578, "*Salmonella Typhimurium/Escherichia Coli* Reverse Mutation Assay (Standard Plate Test and Preincubation Test) with M320102 (Z-Isomer of BAS 320 I) (Including Amendment 1)", (G. Engelhardt and E. Leibold, BASF Experimental Toxicology and Ecology, Ludwigshafen, Germany, Report Project No. 40M0677/014167, 24 January 2005). Triplicate cultures of *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 *uvr*A were exposed to M320102 (96.6% purity), in the presence and absence of S9 mix, at 0 (DMSO), 20, 100, 500, 2500, and 5000 µg/plate for 48 to 72 hours by direct plate incorporation and, subsequently, at 0 (DMSO), 4, 20, 100, 500, and 2500 µg/plate in a preincubation assay (exposure for 20 minutes in flasks followed by plating and exposure for 48 to 72 hours). Precipitation was noted at 100 µg/plate and higher in both assays. The number of revertants per plate was not increased by treatment. Acceptable as supplemental data to BAS 320 I. (Green and Leung, 9/11/06).

53000-0063 222581, 222582, "*Salmonella Typhimurium*/*Escherichia Coli* Reverse Mutation Assay (Standard Plate Test and Preincubation Test) with Reg. No. 4984051 (Metabolite of BAS 320 I)", (G. Engelhardt and E. Leibold, BASF Experimental Toxicology and Ecology, Ludwigshafen, Germany, Report Project No. 40M0670/024150, 7 August 2003 and Amendment (222582), 8 March 2004). Triplicate cultures of *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 *uvr*A were exposed to Reg No. 4984051 (Metabolite of BAS 320 I) (98.7% purity), in the presence and absence of S9 mix, at 0 (DMSO), 20, 100, 500, 2500, and 5000 µg/plate for 48 to 72 hours by direct plate incorporation and, in a second trial, at 0 (DMSO), 62.5, 100, 125, 250, 500, and 1500 µg/plate using the preincubation method (pre-treatment for 20 minutes in flasks followed by plating and exposure for 48 to 72 hours). Precipitation was noted at 500 µg/plate and higher in trial 1 (direct plate incorporation) and at 250 µg/plate and above in trial 2 (preincubation). Cytotoxicity (strain dependent reduction in the number of revertants per plate) was noted at 500 µg/plate and above in trial 1 and at 250 µg/plate and higher in trial 2. No increase in the number of revertants per plate. Acceptable as supplemental data to BAS 320 I. (Green and Leung, 9/11/06).

53000-0063 222583, "*In Vitro* Gene Mutation Test with Reg. No. 4984051 (Metabolite of BAS 320 I) in CHO Cells (HPRT Locus Assay) (Including Amendment 1)", (G. Engelhardt and E. Leibold, BASF Experimental Toxicology and Ecology, Ludwigshafen, Germany, Report Project No. 50M0670/024154, 7 April 2004. Amendment date, 30 November 2004). Duplicate cultures of Chinese hamster ovary cells (substrain K1) were treated with Reg. No. 4984051 (metabolite of BAS 320 I) (98.7% purity) for 4 hours, in the absence of S9 mix, at 0 (DMSO), 0.125, 0.25, 0.5, 1.0, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, or 6.0 μg/ml and, in the presence of S9, at 0 (DMSO), 1.25, 2.5, 3.125, 5.0, 6.25, 10, 12.5, 15, 20, 25, 30, 40, 50, or 100 μg/ml. 4 trials were performed. Expression time was 7 days followed by a 1 week selection period (in medium with 6 thioguanine at 300,000 cells/flask with 12 flasks per treatment group (6 per replicate)). A reduction in the cloning efficiency was noted at 2.5 μg/ml and higher in the absence of S9 and at 20 μg/ml and higher with S9 mix, after the 4-hour exposure period. At the end of the expression period, cytotoxicity was noted at 3 μg/ml and higher without S9 mix and at 30 μg/ml and higher with it. No increase in forward mutations. Acceptable as supplemental data to BAS 320 I. (Green and Leung, 9/12/06).

CHROMOSOME EFFECTS

****53000-0056 222569, 222570**, "*In Vitro* Chromosome Aberration Assay with BAS 320 I in V79 Cells", (G. Engelhardt and E. Leibold, BASF Experimental Toxicology and Ecology, Ludwigshafen, Germany, Report Project No. 32M0071/014157, 26 August 2002. Amendment (222570) date, 22 March 2004). Proliferating Chinese hamster V79 cells were seeded in duplicate (2 chambers of a Quadriperm dish were used per test culture) and exposed (4 hours) to BAS 320 I (96.9% metaflumizone) in the absence of activation at 0 (DMSO), 3.125, 6.25, 12.50, 25, and 50 μg/ml and, in the presence of S9 mix, at 0 (DMSO), 25, 50, and 100 μg/ml. Cells were harvested, fixed (methanol), stained (Giemsa), and evaluated 18 hours after the start of treatment. 100 metaphases from each culture were analyzed for chromosomal aberrations (except where chromosomal damage was evident, then 50 per culture were examined). Mitotic activity was not significantly affected by treatment. Precipitation was visible at 25 μg/ml and higher. A significant increase in the number of aberrant metaphases was recorded in the absence of S9 mix. Clastogenicity was not indicated with activation. Acceptable. (Green and Leung, 9/11/06).

**53000-0056 222571, 222572, "Cytogenetic Study *In Vivo* with BAS 320 I in the Mouse Micronucleus Test After Two Intraperitoneal Administrations", (G. Engelhardt and E. Leibold, BASF Experimental Toxicology and Ecology, Ludwigshafen, Germany, Report Project No. 26M0071/014155, 26 August 2002. Amendment (222572) date, 22 March 2004). 5 male Crl:NMRI mice per group received BAS 320 I (96.9% metaflumizone) twice (with a 24-hour interval) by intraperitoneal injection at 0 (0.5% aqueous carboxymethylcellulose), 500, 1000, and 2000 mg/kg. Bone marrow was sampled 24 hours after the last treatment. Squatting posture was recorded for all animals after each treatment at 500, 1000, and 2000 mg/kg. Additionally, at 2000 mg/kg, piloerection was noted after both treatments and poor general state was recorded 4 hours after the initial dose. 2000 polychromatic erythrocytes were evaluated and scored per animal using a microscope. There was no increase in micronucleated polychromatic erythrocytes. Acceptable. (Green and Leung, 9/11/06).

53000-0063 222584, 222585, "*In Vitro* Chromosome Aberration Assay with Reg. No. 4984051 (Metabolite of BAS 320 I) in V79 Cells", (G. Engelhardt and E. Leibold, BASF Experimental Toxicology and Ecology, Ludwigshafen, Germany, Report/Project No. 32M0670/024151, 16 January 2004 and amendment dated 5 April 2004 (record 222585)). Proliferating Chinese hamster V79 cells were exposed for 4 hours in duplicate (2 chambers of a Quadriperm dish were used per test culture) to Reg. No. 4984051 (metabolite of BAS 320 I) (98.7% purity), in the absence of activation, at 0 (DMSO), 0.25, 0.5, and 1.0 μg/ml and, in the presence of S9 mix, at 0 (DMSO), 1.0, 5.0, 7.5, 10, and 12.5 μg/ml. Cells were harvested and evaluated 18 hours after the start of treatment. 100 metaphases from each culture were evaluated for chromosomal aberrations and 1000 cells per culture were examined for mitotic index determinations. Weak suppression of mitotic activity and a significant increase in the number of aberrant metaphases were noted in the presence of S9 mix. Clastogenicity was not indicated without activation. Acceptable as supplemental data to BAS 320 I. (Green and Leung, 9/12/06).

53000-0063 222586, "Cytogenetic Study *In Vivo* with Reg. No. 4984051 (Metabolite of BAS 320 I) in the Mouse Micronucleus Test After Two Oral Administrations", (BASF Experimental Toxicology and Ecology, Ludwigshafen, Germany, Report/Project No. 26M0670/024161, 3 March 2004). 5 male Crl:NMRI mice per group received two oral gavage doses (24 hours apart) of Reg. No. 4984051 (metabolite of BAS 320 I) (98.7% purity) at 0 (DMSO and olive oil), 500, 1000, and 2000 mg/kg. Bone marrow was sampled 24 hours after the last treatment. No clinical signs of toxicity were recorded in any group after treatment. 2000 polychromatic erythrocytes per animal were microscopically evaluated and scored. No increases in micronucleated polychromatic erythrocytes were indicated. Acceptable as supplemental data to BAS 320 I. (Green and Leung, 9/12/06).

DNA DAMAGE

**53000-0056 222573, 222574, "*In Vivo* Unscheduled DNA Synthesis (UDS) Assay with BAS 320 I in Rat Hepatocytes, Single Oral Administration", (G. Engelhardt and E. Leibold, BASF Experimental Toxicology and Ecology, Ludwigshafen, Germany, Report Project No.

80M0071/014198, 30 June 2003 and amendment (222574), 22 March 2004). 3 male Wistar rats (CrlGlxBrlHan:WI) per group received a single oral gavage treatment of BAS 320 I (96.9% metaflumizone) at 0 (0.5% carboxymethylcellulose), 1000, and 2000 mg/kg. Hepatocytes were sampled 3 and 14 hours post-treatment. 100 cells per animal were evaluated. Net nuclear grain counts were not increased by treatment and cell viability was not affected. Acceptable. (Green and Leung, 9/11/06).

NEUROTOXICITY

53000-0046; 222556; "BAS 320 I - Acute Neurotoxicity Study in Wistar Rats, Single Administered Dose by Gavage (including Amendment 1)"; (U. Kaspers, W. Kaufmann, J. Hellwig; Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen/Rhein, Germany; Project ID. 20S0071/01081; 7/17/03, amended, 1/18/05); Ten Wistar rats/sex/group were dosed orally by gavage with 0, 125, 500 and 2000 mg/kg of BAS 320 I Technical (batch no. PF-01-02-Ve-1-3; purity: 96.9% (E-isomer: 91.0%), (Z-isomer: 5.9%)). The vehicle was aqueous 0.5% carboxymethyl cellulose. No deaths resulted from the treatment. No treatment-related clinical signs were revealed in the functional observational battery or motor activity measurements. No treatment-related lesions were observed in the neuropathology evaluation. **No adverse effect indicated. Acute Neurotoxicity NOEL:** (M/F) > 2000 mg/kg; **Study acceptable.** (Moore, 4/28/06)

53000-0050; 222560; "BAS 320 I - Subchronic Neurotoxicity Study in Wistar Rats: Administration by Gavage for 3 Months"; (U. Kaspers, W. Kaufmann, B. van Ravenzwaay; Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany; Project ID. 51S0071/01069; 3/19/03, amended, 1/18/05); Ten Wistar rats/sex/group (unless otherwise noted) were dosed orally by gavage with 0 (vehicle: aqueous 0.5% methylcellulose), 12, 36, 150 or 300 (males only) mg/kg/day of BAS 320 I Technical (batch no. PF-01-02-Ve-1-3, purity: 96.9%) for 13 weeks. One male and one female in the 150 mg/kg group died on days 59 and 16, respectively. The mean body weights, body weight gain and food consumption of the females in the 150 mg/kg group and males in the 300 mg/kg group were less than that of the control animals ($p<0.01$ or 0.05). These animals also demonstrated increased incidences of discolored feces, piloerection and/or hypothermia. Piloerection was also noted in the functional observational battery as an effect for animals in these groups at various time points during the study. In motor activity assessment, lower numbers of beam interruptions were noted for females in the 150 mg/kg group and males in the 300 mg/kg group at various time points during the study. However, no consistent pattern of effect was elicited by the treatment. No treatment-related lesions noted in the histopathological examination. **No adverse effect evident**. **Neurotoxicity NOEL:** (M) 300 mg/kg/day (based upon the lack of treatment-related neurotoxic effects at the highest dose tested); (F) 150 mg/kg/day (based upon the lack of treatment-related neurotoxic effects at the highest dose tested). **Study acceptable.** (Moore, 6/2/06)

SUBCHRONIC TOXICITY

Rat Subchronic Oral Toxicity Study

53000-0047; 222557; "28-Day/13-Week Oral Toxicity Study in Albino Rats with BAS 320 I"; (J.E. Fischer; BASF Agro Research, Toxicology, Princeton, NJ; Project ID. T-1186; 6/6/02); Five Crl:CD(SD)BR rats/sex/group were dosed orally by gavage with 0 (vehicle: aqueous 0.5% carboxymethyl cellulose), 100, 500 or 1000 mg/kg/day of BAS 320 I technical (lot no. 12136-143; purity: 95.1% (E isomer: 86.7%, Z isomer: 8.4%)) for 4 weeks. An additional 5 animals/sex/group were dosed with 0 or 1000 mg/kg/day of the test material for 13 weeks. The dosage for the high dose animals was changed to 100 mg/kg/day after 7 treatments due to excessive effects on body weight gain and food consumption. No treatment-related deaths occurred during the study. In the 4-week study, the mean body weight and food consumption of both sexes in the 500 and 1000 mg/kg groups was lower than those values for the control ($p<0.05$). For the animals in the 13 week study, the mean body weight and food consumption of the females in the 1000/100 mg/kg group were lower throughout the treatment period ($p<0.05$). No treatment-related effects were noted in the hematology evaluation. In the clinical chemistry, after 4 weeks of treatment, the serum cholesterol levels were elevated for the 1000 mg/kg males and for the females in all of the

treatment groups ($p<0.05$). The serum albumin was increased for males in all of the treatment groups after 4 weeks ($p<0.05$). In the necropsy examination, the mean relative liver weights of the 500 and 1000 mg/kg males were increased over that of the controls after 4 weeks of treatment ($p<0.05$). The mean relative adrenal weight of the 1000 mg/kg males was increased as well ($p<0.05$). The mean relative ovaries/oviduct weight of the 1000 mg/kg females was lower than that of the control ($p<0.05$). There was no affect on the organ weights of the animals treated for 13 weeks. In the histopathology examination, the centrilobular hypertrophy of the liver was noted for both sexes in the 500 and 1000 mg/kg after 4 weeks of treatment (males and females, 0: 0/5 vs. 500: 2/5, 1000: 4/5). Extramedulary hematopoiesis was evident in the spleen of both sexes in the 500 and 1000 mg/kg groups ((M), 0: 0/5 vs. 500: 2/5, 1000: 3/5, (F) 0: 0/5 vs. 500: 3/5, 1000: 5/5). Decreased cyclic activity in the ovaries and uterus was noted for the females in the 500 and 1000 mg/kg groups (0: 0/5 vs. 500: 2/5, 1000: 3/5). Target organ: liver; **No adverse effect indicated. Subacute Oral Toxicity NOEL:** (M/F) 100 mg/kg/day (based upon the liver hypertrophy and extramedullary hematopoiesis in the spleen noted for both sexes in the 500 mg/kg treatment group); **Study supplemental.** (Moore, 5/2/06)

Rat Subchronic Oral Toxicity Study

53000-0059; 222577; "M320I02 (Z Isomer of BAS 320 I) - Subchronic Toxicity Study in Sprague-Dawley Rats; Administration by Gavage for 3 Months"; (U. Kaspers, K. Deckardt, K. Kuttler, J. Hellwig; Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen/Rhein, Germany; Project ID. No. 51S0677/01087; 4/16/04); Ten Sprague-Dawley rats/sex/group were dosed orally by gavage with 0 (vehicle: 0.5% carboxymethyl cellulose), 100, 300 or 1000 mg/kg of M320I02 (Z-isomer of BAS 320 I) (batch no. 01893-152; purity: 99.7%) for 13 weeks. One female each died in the 300 and 1000 mg/kg treatment groups, possibly due to treatment-related effects. The females in the 1000 mg/kg group demonstrated a lower mean body weight towards the latter part of the study (NS). Mean food consumption was lower for the 300 and 1000 mg/kg females at various times during the study ($p<0.05$). No treatment-related effect was noted in the hematology, ophthalmology or clinical chemistry. The FOB did not reveal any treatment-related effects. The motor activity of both sexes in the 1000 mg/kg group and of the females in the 300 mg/kg group was less than that of the controls ($p<0.05$ or 0.01). In the necropsy examination, the mean relative liver weights of the females in all of the treatment groups were greater than that of the controls ($p<0.05$ or 0.01). However, no histopathological effects were associated with this increased weight. The mean relative adrenal gland weight of the females in the 300 and 1000 mg/kg groups were greater than that of the controls ($p<0.05$). In the histopathological examination, the incidence of centrilobular hypertrophy (grade 1) was noted in the livers of the treated males (0: 0/10 vs. 100: 1/10, 300: 2/10, 1000: 4/10). For the females, the 1000 mg/kg group demonstrated decreased cellularity in the spleen (0: 0/10 vs. 1000: 2/10). The females in the 300 and 1000 mg/kg groups exhibited a greater severity of vacuolation in the adrenal gland (grade 4 and 5 designation, 0: 1/10 vs. 300: 8/10, 1000: 8/10). Lymphocyte necrosis was noted in the mesenteric lymph node of the females in the 300 and 1000 mg/kg groups (0: 0/10 vs. 300: 1/10, 1000: 3/10). **Possible adverse effect:** lymphocyte necrosis in the mesenteric lymph node; **Subchronic Oral Toxicity NOEL:** (M/F) 100 mg/kg/day (based upon the hypertrophy noted in the livers of the 300 mg/kg males and the treatment-related effects on the lymphocytes in the mesenteric lymph nodes and on the adrenal glands of the 300 mg/kg females); **Study acceptable.** (Moore, 7/7/06)

Rat Subchronic Oral Toxicity Study

53000-0062; 222580; "Reg. No. 4984051 (Metabolite of BAS 320 I) - Subchronic Toxicity Study in Sprague-Dawley Rats; Administration by Gavage for 3 Months"; (U. Kaspers, K. Deckardt, W. Kaufmann, J. Hellwig; Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen/Rhein, Germany; Project ID. No. 50S0670/02058; 4/16/04); Ten Sprague-Dawley rats/sex/group were dosed orally by gavage with 0 (vehicle: 0.5% carboxymethyl cellulose), 50, 200 or 1000 mg/kg/day of Reg. No. 4984051 (Metabolite of BAS 320 I) (batch no. WI20555, purity: 98.7%) for 13 weeks. The males in the 200 and 1000 mg/kg groups demonstrated lower mean body weights by the termination of the study (NS). Although the males in the 200 and 1000 mg/kg groups exhibited an increased number of rearings in the open field evaluation of the

Functional Observational Battery ($p<0.01$), no other correlative parameters were affected by the treatment. In the urinalysis, the females in the 1000 mg/kg group had increased level of leucocytes in the sediment. However, no pathological effects were noted on the kidneys. The hematology, clinical chemistry and ophthalmological examinations did not reveal any treatment-related effects. In the necropsy examination, the mean absolute adrenal weights and mean relative liver and adrenal weights of the 200 and 1000 mg/kg females were greater than those of the controls ($p<0.05$ or 0.01). An increased incidence of central hypertrophy was noted in the livers of both sexes in the 1000 mg/kg group ((M) 0: 3/10 vs. 1000: 9/10, (F) 0:1/10 vs. 1000: 6/10). There was an increased incidence in tubular hyperplasia in the kidneys of the males in the 1000 mg/kg group (0: 0/10 vs. 1000: 3/10). An increased incidence of follicular cell hypertrophy was noted for the males in all of the treatment groups (0: 2/10 vs. 50: 6/10, 200: 7/10, 1000: 9/10). However, upon closer examination, it was concluded that the incidence reported for the control group animals was low in comparison to the results noted in another study. Hemosiderosis and extra-medullary hematopoiesis was noted in the spleen of the 1000 mg/kg females (hemosiderosis, 0: 1/10 vs. 1000: 3/10, hematopoiesis, 0: 0/10 vs. 1000: 3/10). **No adverse effect noted. Subchronic Oral Toxicity NOEL:** (M/F) 200 mg/kg/day (based upon the increased incidence of central lobular hypertrophy in the liver of the 1000 mg/kg treated animals). **Study acceptable.** (Moore, 7/10/06)

Rat Subchronic Dermal Toxicity Study

53000-0049; 222559; "BAS 320 I - Subchronic Toxicity Study in Wistar Rats, Dermal Application for 3 Months"; (U. Kaspers, K. Deckhardt, S. Burkhardt, B. van Ravenzwaay; Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany; Project ID. 33S0071/01067; 1/27/04); The skin of 10 Wistar rats/sex/group was exposed to 0 (vehicle: aqueous 0.5% methylcellulose), 100, 300 or 1000 mg/kg/day of BAS 320 I Technical (test substance no. 01/0071-2, purity: 96.9%) for 6 hours/day, 5 days/week for 3 months. No deaths resulted from the treatment. Both sexes in the 300 and 1000 mg/kg groups demonstrated reduced food consumption and lower mean body weight grain over the course of the study (NS, $p<0.05$ or 0.01). No treatment-related effects were noted in the ophthalmology, hematology or urinalysis examinations. The calcium, sodium and inorganic phosphate levels were elevated for the 1000 mg/kg females ($p<0.05$ or 0.01). The mean serum cholesterol levels were increased for the 300 and 1000 mg/kg females ($p<0.05$ or 0.01). In the necropsy examination, the mean relative liver weight of the 1000 mg/kg females was increased over that of the controls ($p<0.01$). In the histopathology examination, an increased incidence and severity of lymphocytic necrosis was noted in the spleen of the 1000 mg/kg females (0: 3/10 vs. 1000: 6/10). Reduction of the periartiolar lymphoid sheath (PALS) was noted in the spleen of 3 of the females in the 1000 mg/kg group. In the thymus, there was increased incidence and severity of starry sky cells in the females of the 300 and 1000 mg/kg groups (0: 5/10 vs. 300: 8/10, 1000: 9/10). Lymphocytic necrosis was noted in the mesenteric lymph nodes of the 300 and 1000 mg/kg females (0: 0/10 vs. 300: 3/10, 1000: 4/10). Atrophy of the mandibular lymph nodes was noted for the 300 and 1000 mg/kg females (0: 0/10 vs. 300: 2/10, 1000: 2/10). Vacuolation of the zona fasciculata in the adrenal cortex was noted for the 1000 mg/kg females (0: 0/10 vs. 1000: 6/10). **Possible adverse effect:** lymphocytic necrosis. **Subchronic Dermal Toxicity:** (M/F): 100 mg/kg/day (based upon reduced body weight gain for both sexes in the 300 mg/kg treatment group and the incidence and/or severity of lesions in the lymph nodes of the females of this group); **Study acceptable.** (Moore, 5/12/06)

METABOLISM

53000-0057; 222575; "BAS 320 1 (AC 836519): Absorption, Distribution, Metabolism, Excretion, and Pharmacokinetics of [Benzonitrile ring-U-^{14}C] and [Trifluoromethoxyphenyl ring-U-^{14}C] BAS 320 I in the Rat"; (J. Afzal, J. Zulalian; XenoBiotic Laboratories (XBL), Plainsboro, NJ; Project ID. No. MET 02-012; 9/30/02); Three to five Sprague-Dawley rats/sex/group (time point) were dosed orally by gavage with [benzonitrile ring-U-^{14}C + tolunitrile-benzyl-^{13}C] BAS 320 I **(1)** (lot no. AC 12432-36, chemical purity: 99.76%, radiochemical purity: 97.98%, specific activity: 13.73 uCi/mg), **(2)** (lot no. AC 12694-6, chemical purity: 98.27%, radiochemical purity: 97.745, specific activity: 0.98 uCi/mg), **(3)** lot no. AC 12694-90, chemical purity: 100.1%, radiochemical

purity: 96.6%, 8.0 uCi/mg, [trifluoromethoxyphenyl ring-U-^{14}C + trifluoromethoxyphenylamine-^{15}N] BAS 320 I **(4)** (lot no. AC 12432-42, chemical purity: 100%, radiochemical purity: 98.42%, specific activity: 12.72 uCi/mg), **(5)** (lot no. AC12694-8, chemical purity: 97.92%, radiochemical purity: 97.68%, specific activity: 0.98 uCi/mg), or [trifluoromethoxyphenyl ring-U-^{14}C + trifluoromethoxyphenyl ring-C_1-^{13}C] BAS 320 I **(6)** (lot no. AC 12694-91, chemical purity: 97.1%, radiochemical purity: 97.6%, specific activity: 8.0 uCi/mg) at concentrations of 30 or 1000 mg/kg. Compound No. 1 was used for the 30 mg/kg treatments in the pharmacokinetic, biliary excretion, mass balance and tissue-organ distribution studies. Animals were dosed with 1000 mg/kg of Compound No. 2 in the pharmacokinetic, biliary excretion, mass balance and tissue-organ distribution studies. Animals were dosed 14 times with 30 mg/kg/day of Compound No. 3 in the accumulation study. Compound No. 4 was used to treat animals with 30 mg/kg in the pharmacokinetic, biliary excretion, mass balance and tissue-organ distribution studies. Animals were dosed with 1000 mg/kg of Compound No. 5 in the pharmacokinetic, biliary excretion, mass balance and tissue-organ distribution studies. Animals were dosed 14 times with 30 mg/kg/day of Compound No. 6 in the accumulation study. The pharmacokinetic study demonstrated tmax values which ranged from 10 to 23 hours for the 30 mg/kg treatment level depending on the position of the labeling and the sex of the animals. For the 1000 mg/kg treatment level, the tmax values ranged from 23 to 48 hours. The Cmax levels were higher for the animals treated with Compound Nos. 4 and 5 than for Compound Nos. 1 and 2. The elimination half lives ranged from 38 to 48 hours for the [benzonitrile ring-U-^{14}C + tolunitrile-benzyl-^{13}C] BAS 320 I treated animals and from 139 to 402 hours for the [trifluoromethoxyphenyl ring-U-^{14}C + trifluoromethoxyphenylamine-^{15}N] BAS 320 I treated animals which corresponded to the greater rate of clearance and shorter residence time. The volume of distribution was greater for the animals treated with [benzonitrile ring-U-^{14}C + tolunitrile-benzyl-^{13}C] BAS 320 I than for those that were treated with [trifluoromethoxyphenyl ring-U-^{14}C + trifluoromethoxyphenylamine-^{15}N] BAS 320 I. The mass balance study revealed that the radiolabel was predominantly recovered in the feces of both sexes for both compound moieties and both treatment levels (90 to 112% of the administered dose). Recovery in the urine comprised only 0.6 to 1.8% of the administered dose. The biliary excretion study demonstrated that another 0.2 to 4.7% of the administered dose was recovered in the bile depending on the treatment level and compound moiety. The overall absorption level of the test material was ascertained to range from 0.7 to 7.2% of the administered dose. Among the tissues surveyed, fat was the primary site from which radiolabel was recovered. The level of radioactivity persisted in the fat up to 12 days post-dose. When the animals were treated with [trifluoromethoxyphenyl ring-U-^{14}C + trifluoromethoxyphenylamine-^{15}N] BAS 320 I, the level of radioactivity persisted in the red blood cells up to 12 days post-dose. This phenomenon was not observed when the animals were treated with [benzonitrile ring-U-^{14}C + tolunitrile-benzyl-^{13}C] BAS 320 I. When the animals were treated with multiple doses of the test material, levels of radiolabel recovered from all of the surveyed tissue were elevated over those levels observed at the same time post-final dose for the animals receiving a single dose. These results were a consequence of the relatively long elimination half-life for the test material. This phenomenon was apparent up to 12 days post-final dose. The radiolabeled moieties recovered from the bile and the urine indicated that hydroxylation of the aniline or benzonitrile rings and hydrolysis of the hydrazine carboxamide group were common sites of metabolism. Further conjugation of the ring hydroxylation sites with sulfate or glucuronide was also noted. Malonic and oxalic acid were shown to form conjugates with trifluoromethoxyaniline. The carboxyl group of the cyanobenzoic acid conjugated with glycine. The formation of a gluthathione conjugate resulted from the displacement of the one of the fluorine atoms on either the trifluoromethyl or trifluoromethoxy moieties. **Study acceptable.** (Moore, 7/5/06)